Workshop F
Lymphokines and their Receptors, Effector Mechanisms

Ruhr-Universität Bochum, AG Infektabwehr, D-4630 Bochum, FRG

F.1 Modulation of CD23 expression, sCD23 and IgE synthesis from peripheral blood lymphocytes by glucocorticoids and signal transduction modulators

A. Fischer, E. Weinholz, and W. König

We studied the influence of Prednisolone (GC) and signal transduction modulators on IgE synthesis and CD23 expression. Stimulation of PBL from normal and atopic donors with GC (10^{-8}M) led to an enhanced IgE synthesis. Costimulation with GC and IL-4 led to a further enhancement of the IgE synthesis. For the induction of IgE synthesis it was sufficient to add GC prestimulated T cells. To analyze the effect of GC on cellular interactions, T and B cells were costimulated in the Transwell system. Under these conditions, GC prestimulated T cells enhanced IgE synthesis, indicating the modulation of the cytokine expression pattern of T cells after preactivation with GC. The analysis of CD23, which is involved in IgE regulation, revealed that the spontaneous and IL-2 induced CD23 expression on B cells is suppressed by GC, whereas the IL-4 induced CD23 expression on B and T cells remains unaffected. The sCD23 release is suppressed completely by GC concentrations less than 10^{-8} M. These results indicate that GC acts directly on the B-cell differentiation process. Since IgE synthesis and CD23 expression seemed to be regulated in coordinated fashion by cytokines, the effect of signal transduction modulators and lipid mediators on the cytokine induced CD23 expression was analyzed. On PBL, dibutyryl-cAMP enhances the IL-4 induced CD23 expression, whereas the IL-2 induced CD23 expression is suppressed. LTA4, LTB4, and LTA4-methylester are able to modulate the IL-4 induced CD23 expression. These data suggest that IL-4 and IL-2 display their CD23 enhancing effect via different signal transduction pathways.

Institut für Klinische Mikrobiologie, Wasserturmstr. 3, 8520 Erlangen, FRG

F.2 Identification of two forms of the murine interleukin-1β precursor

C. Günther, B. Spriewald, C. Nicklas, M. Rollinghoff, and H. U. Beuscher

Interleukin-1β (IL-1β) is synthesised as a precursor protein (31 kD), lacking a secretory leader sequence, required for protein translocation. Consistent with this observation, IL-1β

2028902087

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Workshop F
Lymphokines and their Receptors, Effector Mechanisms

Ruhr-Universität Bochum, AG Infektabwehr, D-4630 Bochum, FRG

F.1 Modulation of CD23 expression, sCD23 and IgE synthesis from peripheral blood lymphocytes by glucocorticoids and signal transduction modulators

A. Fischer, E. Weinholz, and W. König

We studied the influence of Prednisolone (GC) and signal transduction modulators on IgE synthesis and CD23 expression. Stimulation of PBL from normal and atopic donors with GC (10^{-8}M) led to an enhanced IgE synthesis. Costimulation with GC and IL-4 led to a further enhancement of the IgE synthesis. For the induction of IgE synthesis it was sufficient to add GC prestimulated T cells. To analyze the effect of GC on cellular interactions, T and B cells were costimulated in the Transwell system. Under these conditions, GC prestimulated T cells enhanced IgE synthesis, indicating the modulation of the cytokine expression pattern of T cells after preactivation with GC. The analysis of CD23, which is involved in IgE regulation, revealed that the spontaneous and IL-2 induced CD23 expression on B cells is suppressed by GC, whereas the IL-4 induced CD23 expression on B and T cells remains unaffected. The sCD23 release is suppressed completely by GC concentrations less than 10^{-8} M. These results indicate that GC acts directly on the B-cell differentiation process. Since IgE synthesis and CD23 expression seemed to be regulated in coordinated fashion by cytokines, the effect of signal transduction modulators and lipid mediators on the cytokine induced CD23 expression was analyzed. On PBL, dibutyryl-cAMP enhances the IL-4 induced CD23 expression, whereas the IL-2 induced CD23 expression is suppressed. LTA4, LTB4, and LTA4-methylester are able to modulate the IL-4 induced CD23 expression. These data suggest that IL-4 and IL-2 display their CD23 enhancing effect via different signal transduction pathways.

Institut für Klinische Mikrobiologie, Wasserturmstr. 3, 8520 Erlangen, FRG

F.2 Identification of two forms of the murine interleukin-1β precursor

C. Günther, B. Spriewald, C. Nicklas, M. Röllinghoff, and H. U. Beuscher

Interleukin-1β (IL-1β) is synthesised as a precursor protein (31 kD), lacking a secretory leader sequence, required for protein translocation. Consistent with this observation, IL-1β

202890208

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

has never been detected within membranes of the endoplasmatic reticulum. Therefore the intracellular pathway(s) leading to extracellular IL-1β remain unknown.

We have investigated the synthesis of IL-1β by murine macrophages. SDS-PAGE under non-reducing conditions revealed two forms of cell-associated IL-1β: a 31 kD polypeptide and a 35 kD polypeptide. When analyzed under reducing conditions both forms migrated as one band (i.e. 35 kD). Several lines of evidence indicate that the faster migration of individual IL-1β precursor molecules (i.e. 31 kD) is due to intrachain disulfide bonding: (1) Fully reduced 31 kD IL-1β polypeptides efficiently refolded if the redox potential was adjusted to favor reformation of disulfides. (2) Refolding of the 31 kD precursor peptide could be blocked by alkylation with iodoacetamide. (3) Alkylation of IL-1β was increased after reduction of the protein.

Since disulfide bonds are usually not detected in cytosolic proteins, because the high content of glutathione would brake such S-S bridges, our data indicate that the two forms of the IL-1β precursor are present in different cellular compartments. Co-localization of IL-1β with known marker proteins for intracellular membranes and organelles is in progress to definitively characterize the intracellular localization of IL-1β precursor molecules.

[1]Dept. of Immunology and Transfusion Medicine, Medical University of Hannover, 3000 Hannover, FRG, [2]Dept. of of Pharmaceutical Research, Ciba Geigy Ltd, Basel, Switzerland

F.3 Correlation of the antiproliferative effect and the Mx-homologue induction by IFNs in patients with malignant melanoma

D. Jakschies[1], H. K. Hochkeppel[2], M. A. Horisberger[2], H. Deicher[1], and P. von Wussow[1]

Interferons (IFNs) are produced by eukaryotic cells in response to a number of stimuli such as antigens, mitogens and viral infections. They induce a number of intracellular proteins, which are believed to be responsible for the antiviral activity, cell growth inhibition and a variety of other biological effects. One of the IFN-induced proteins is the human Mx-homologous protein, which is most likely identical with the human Mx-A-protein. Three of six melanoma cell lines produced this intracellular human Mx-homologue upon incubation with IFN-α or IFN-γ, yet all six melanoma cell lines tested were susceptible to the antiproliferative effect of rIFN-α and rIFN-γ. Compared per antiviral unit IFN-γ had a weaker Mx-inducing, but a stronger antiproliferative activity than rIFN-α. These data demonstrate that the IFN-induced Mx-homologous protein is not involved in the antiproliferative action of IFNs on malignant melanoma cell lines.

Furthermore, 51 patients with advanced malignant melanoma were treated thrice weekly with 10×10^6 IU rIFN-α2b and 6×10^6 nIFN-α, respectively. Nine of the 51 patients experienced systemic objective tumor responses (3 CR, 6 PR), but had Mx-concentrations in their PBLs equal to the Mx-levels of non-responder during IFN-α-therapy. Therefore, the level of the Mx-homologous protein induced during IFN-therapy is not a predictive marker for an antitumor response in malignant melanoma.

2028902089

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Institute of Immunology and Genetics, German Cancer Research Center, D-6900 Heidelberg, FRG

F.4 Extracellular ATP modulates tumor necrosis factor (TNF) induced lysis of L929 cells

Doris Kinzer and Volker Lehmann

Extracellular ATP in concentrations of 1.0–2.5 mM inhibit TNF induced cytolysis of L929 cells. To be inhibitory ATP has to be added together with TNF and must be present throughout the entire assay period (24–48 h). In contrast, limited exposure of TNF treated L cells to ATP leads to a substantial enhancement of the TNF induced cytolysis. Both ATP effects are specific. Thus, ATP acts by itself without being first converted into adenosine, which is also capable of modulating TNF responses. Moreover, a nonhydrolyzable ATP analogue AMP-PCP is almost as reactive as ATP, whereas ADP is less reactive and GTP is nonreactive. The inhibitory effect of ATP on TNF induced cytolysis is controlled by the adenosine receptor. After its downregulation with N^6-(R-phenylisopropyl) adenosine (R-PIA) inhibition no longer occurred. Instead, a substantial enhancement of the TNF induced cytolysis by ATP was observed. The results suggest that the TNF induced cytolysis of L929 cells is modulated by interactions of external ATP with ectozymes on the surface of the plasma membrane.

[1]Lehrstuhl für Med. Mikrobiologie und Immunologie, AG Infektabwehr, Ruhr-Universität Bochum, [2]Abteilung für Verbrennungskrankheiten, BG Klinikum „Bergmannsheil", 4630 Bochum, FRG

F.5 Functional alterations of granulocytes from severely burned patients

Manfred Köller[1], Joachim Brom[1], Sabine Kasimir[1], Fritz E. Müller[2], and Wolfgang König[1]

Patients suffering from severe burns acquire various alterations of their immune functions. We investigated the generation and metabolism of leukotrienes (LTs), monoeicosatetraenoic acids (HETEs) and platelet-activating factor (PAF) from granulocytes (PMNs) of severely burned patients. The stimulation (Ca-ionophore A23187, opsonized zymosan, fMLP, NaF) of patients' cells led to a decrease in leukotriene- and HETE-generation as compared to normal donor cells. The onset of this PMN-dysfunction correlated with the onset of invasive microbial growth. We observed an enhanced LTB_4-metabolism, a decreased LTB_4-receptor expression and alterations in the availability of free arachidonic acid from the phospholipid structures. The metabolism of PAF and lyso-PAF was decreased in patients' granulocytes. Hyperthermic treatment of PMNs from normal donors could partly mimic the observed PMN-dysfunctions of severely burned patients. Furthermore, an increase in heat-shock protein generation was obtained in leukocytes from severely burned patients as was analyzed by Western blotting techniques. These data indicate that the stress response ("heat-shock response") may be involved in burn-induced cellular dysfunctions.

20289020090

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Institute of Immunology and Transfusion Medicine, University of Lübeck, Medical School, 2400 Lübeck, FRG

F.6 Interferon and lymphokine production of human placental cells and cord blood cells

Andrea Kruse, Lothar Rink, Inge Rutenfranz, and Holger Kirchner

The placenta plays an important role in protecting the fetus from maternal infections. Human placental tissue contains a large number of immunologically active mononuclear cells. We compared lymphokine production of human placental leukocytes with that of adult and cord blood leukocytes. There was a defect of interferon (IFN) gamma production after stimulation with phytohemagglutinin (PHA) of lymphocytes from cord blood and fetal tissue. Decidual cells incubated with PHA synthesized higher levels of IFN-gamma comparable to IFN-gamma levels of adult blood leukocytes. Mononuclear phagocytes, isolated from placenta and cord blood, were found to produce various cytokines such as interleukin-6 (IL-6) and interleukin-1 (IL-1). LPS-stimulated cord blood cells synthesized less IL-6 than adult blood cells but more than LPS-activated placental cells. Cord blood monocytes were also producers of tumor necrosis factor (TNF) and IL-1, especially IL-1 beta. Collectively, these results indicate that T lymphocytes from fetal tissue and cord blood are functionally immature. However, fetal mononuclear phagocytes and adult macrophages produce comparable amounts of various cytokines such as IL-6 and IL-1.

Department of Clinical Immunology, Department of Hematology, Medizinische Hochschule Hannover, 3000 Hannover, FRG, Eurocetus, Amsterdam, Netherlands

F.7 *In vitro* and *in vivo* biological effects of rIL-2 in advanced multiple myeloma

R. Leo, D. Peest, R. Hein, S. Stannat, S. Quesada, J. Atzpodien, H. Kirchner, C. R. Franks, P. A. Palmer, M. P. J. Evers, and H. Deicher

Studies on immunoregulation of myeloma tumor cells indicate that the monoclonal immunoglobulin production of cultured myeloma bone marrow cells is suppressed by purified autologous T cells.[1] This downregulation could be enhanced *in vitro* by stimulation of T cells with anti-CD3-antibodies as well as by the addition of rIL-2 to the bone marrow cultures in 14 out of 20 cases in a dose-dependent manner.

Based on these data we initiated a phase I/II pilot study to evaluate the biological effects of rIL-2 *in vivo*. So far we have treated 7 patients with s.c. rIL-2 (twice daily for 2 days followed by weekly administrations of on tenth of the original dose twice daily during 5 subsequent days until day 56). At present all patients show at least stable disease during therapy.

Concurrent cell surface marker analyses and functional tests were performed on PBMC; serum levels of IL-2, soluble IL-2 receptors and anti-IL-2 antibodies were estimated. A lymphocyte population bearing CD3, CD4 and – in high density – CD25 markers, appeared during therapy. In parallel, increased *in vitro* cytotoxic functions could be observed. These data show, that low dose s.c. rIL-2 regularly leads to lymphocyte activation *in vivo*.

[1] Peest, D., I. de Vries, R. Hölscher, R. Leo, H. Deicher, 1989. Effect of IL-2 on the *ex vivo* growth of human myeloma cells, Cancer Immunol Immunother 30: 227–232.

2028902091

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Institute of Immunology, Philipps University, 3550 Marburg, FRG

F.8 Inhibition of TNF-α production by microtubuli disrupting drugs

Z. Li, C. Mohr, and D. Gemsa

Microtubuli-binding drugs such as colchicine, vinblastine or vincristine have recently been shown by us to inhibit the lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNF-α) production of macrophages. It remained unknown, however, whether colchicine and related compounds would affect TNF-α production by interference with transcriptional, translational or secretory processes. In the present study, rat peritoneal macrophages were used to examine the effect of colchicine on microtubuli-dependent secretory systems. In the presence of colchicine (10^{-5}M), LPS-induced TNF-α release was suppressed but an intracellular TNF-α accumulation was not noted which indicated that colchicine-blocked transport systems were not responsible for diminished TNF-α release. A further kinetic analysis on the molecular level revealed that LPS-induced TNF-α mRNA accumulation was partially suppressed by colchicine treatment (20% by 2 hrs, 40% by 3.5 hrs). In contrast, lumicolchicine, a microtubulus-inactive derivative, was not suppressing TNF-α production. These data show that colchicine and related drugs may not inhibit TNF-α production on the secretory level but earlier on biosynthetic processes involved in transcription/translation. Thus, microtubulus-dependent steps may also play a role in afferent or efferent signal transfer during TNF-α gene expression and intracellular TNF-α mRNA transport.

[1]Institute of Immunology and Transfusion Medicine, Medical University of Lübeck, Medical School, Lübeck, FRG, and [2]Institute of Virus Research, German Cancer Research Center, Heidelberg, FRG

F.9 Induction of lymphokines in human spleen cell cultures by the *Mycoplasma arthritidis* derived mitogen MAS

Lothar Rink[1], Andrea Kruse[1], Werner Nicklas[2], Jörg Hohfeld[2], and Holger Kirchner[1]

In cultures of *Mycoplasma arthritidis* a potent T-cell mitogen, MAS, is produced. Stimulation by MAS is MHC class II-restricted, and only mouse strains expressing an intact I-E-alpha-molecule in the MHC class II complex react to MAS. We found that MAS induced interferon(IFN)-gamma in murine spleen cells and interleukin-6 (IL-6) in murine bone marrow derived macrophages. Furthermore, MAS induced IFN-gamma and interleukin-2 (IL-2) in human peripheral blood lymphocytes. We also investigated human spleen cell cultures with respect to the production of these lymphokines. In human spleen cell cultures MAS induced interleukin-1 (IL-1) beta synthesis within 8 h and there was a maximum of IL-1 beta secretion between 36 h and 72 h, whereas interleukin-1 alpha could not be detected. In these cultures IL-2 was first detected after 12 h and had a maximum after 72 h. In the same cultures the concentration of interleukin-4 (IL-4) increased from 24 h to 72 h similar to the kinetic of IFN-gamma secretion. The previously reported fact that IL-4 inhibited IFN-gamma production could be the reason why MAS induced only small amounts of IFN-gamma whereas it is a very potent T-cell mitogen. Collectively, our results indicate that MAS induces a network of lymphokine interactions in human spleen cell cultures.

2028902092

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Diabetes Research Institute, Heinrich Heine University, Düsseldorf, FRG

F.10 Hypersecretion of TNF-α in diabetes prone BB-rats

Helga Rothe, Karin Fehsel, and H. Kolb

Tumor necrosis factor (TNF)-α is possibly involved in the pathogenesis of type I diabetes because of the essential contribution of the pancreatic islet inflammation. We therefore studied the production of TNF-α in diabetes prone (dp) BB-, diabetes resistent (dr) BB- and Wistar-rats. Peritoneal cells were isolated 4 days after *in vivo* activation with *C. parvum* and the adherent cell fraction stimulated with LPS *in vitro*. TNF-α secretion was detected by a standard bioassay with L929 cells. Peritoneal macrophages of dp BB-rats secreted strikingly higher amounts of TNF-α (1300 U/10^6 cells) than of dr BB-rats and Wistar-rats (40 U/10^6 cells and 52 U/10^6 cells, respectively). This aberrant TNF-α production was seen prior to insulitis. In addition bone marrow derived macrophages of dp BB-rats showed enhanced TNF-α secretion, indicating a defect at the level of precursor cells. Crosses between dp BB-rats and Wistar-rats revealed that the enhanced TNF-α production is a recessive trait. These data show a defect of macrophages and macrophage precursor cells in the dp BB-rats. The upregulated production of TNF-α may have an impact on macrophage-T-cell interactions and on islet inflammation.

[1]Lehrstuhl für Medizinische Mikrobiologie und Immunologie, AG Infektabwehr, Ruhr-Universität Bochum, [2]Abteilung für Verbrennungskrankheiten, BG Klinikum „Bergmannsheil", 4630 Bochum, FRG

F.11 T- and B-lymphocyte dysfunctions in severely burned patients

Bernhard Schlüter[1], Fritz E. Müller[2], and Wolfgang König[1]

We studied phenotypical and functional parameters of peripheral blood lymphocytes from 10 severely burned patients (burn injuries ranging from 25 % up to 72 % TBSA). The absolute number and the relative proportion of T-, B-, and O-cells remained unchanged post burn. In contrast, mitogen (Con A,PHA, PWM, SAC)-induced T- and B-cell blastogenesis revealed a triphasic kinetic pattern: while the proliferation reached normal or even enhanced levels at early and late time intervals, it was suppressed during the second to fourth/fifth week *post burn*. This time course was also obtained for the PWM- or SAC-induced IgM synthesis whereas IgG synthesis was substantially reduced over the total period monitored (up to 50 days *post burn*). T cells were highly activated in the posttraumatic course as assessed by the spontaneous and IL-2-induced expression of CD25. Concomitantly, the cytokine (IL-4, IL-2)-induced B-cell activation was markedly suppressed as determined by the CD23 expression. In addition, the release of the soluble CD23 which serves as an autocrine B-cell growth factor was reduced. These findings indicate that severely burned patients acquire T- and B-lymphocyte dysfunctions which refer to activation, proliferation, and differentiation processes and thus contribute to the increased susceptibility to infection and sepsis.

2028902093

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Institut für Immunologie, Obere Zahlbacher Str. 67, D-6500 Mainz, FRG

F.12 Characterization of a T cell-derived lymphokine which enhances the proliferation of murine mucosal mast cell lines induced by a combination of IL-3 and IL-4

E. Schmitt, C. Hüls, B. Nagel, R. van Brandwijk, and E. Rüde

Bone marrow-derived mucosal mast cells (BMMC) were shown to proliferate *in vitro* in response to interleukin (IL)-3. Furthermore, we and others demonstrated that IL-4 synergistically enhances the IL-3-induced growth of freshly isolated and short term-cultured BMMC without having any growth promoting activity for this cell type by itself.

In this study we showed that another soluble factor, provisionally termed mast cell costimulatory activity (MCA), enhances the proliferation of BMMC induced by a combination of IL-3 and IL-4. MCA exerts its biological activity, concerning mast cell proliferation, only in the presence of IL-3 and IL-4. In combination with either IL-3 or IL-4 alone MCA has no effect on the growth of BMMC which was confirmed using neutralizing concentrations of an anti-IL-3 antiserum and a monoclonal anti-IL-4 (11B11) antibody. Partial purification of MCA illustrates that it can be separated from IL-2, IL-3, IL-4, IL-5, IL-6, IL-9 and GM-CSF. By mixing experiments with a large panel of lymphokines it could further be demonstrated that MCA cannot be identical with IL-1–IL-7, IL-9, G-, M-, GM-CSF, TNF-α, TNF-β, and IFN-γ. MCA was found to be relatively resistant to digestion with trypsin, since about six hours of incubation were required for complete inactivation, whereas the biological activity of IL-2, IL-3, and IL-4 were destroyed within 30 minutes.

Finally, it was found that MCA is preferentially produced by activated T_H2 cells. Thus MCA appears to be a novel T-cell derived lymphokine which, in concert with IL-3 and IL-4, promotes the proliferation of T cell-dependent BMMC.

Supported by the BMFT, grant 01KC 89033.

[1]Institut für Immunologie, Obere Zahlbacher Str. 67, D-6500 Mainz, FRG, [2]Institut Ludwig, Avenue Hippocrate 74, UCL 7459, B-1200 Brussels, Belgium

F.13 IL-4 enhances the production of IL-9

E. Schmitt[1], R. van Brandwijk[1], J. van Snick[2], and E. Rüde[1]

$CD4^+$ T cells can be subdivided into two populations (T_H1, T_H2) based on the panel of lymphokines which they secrete after activation. We have found that PPD-specific T-cell lines developed towards a T_H1 like phenotype if IL-2 was used for the establishment of these lines and a T_H2 like phenotype if IL-4 was employed.

In order to elucidate the mechanism of action of IL-2 and IL-4 on T-cell development we investigated the secretion of different cytokines, among them IL-9, by T-cells under the influence of IL-2 or IL-4. IL-9 is a T-cell derived lymphokine which was recently cloned in mouse and man. It has been demonstrated that IL-9 stimulates the proliferation of certain long term cultured T-cell lines. In addition it was shown that this lymphokine promoted IL-6 production of several mast cell lines both in the presence or absence of IL-3/IL-4 and it enhanced the IL-3 induced proliferation of these lines. We found that IL-4 substantially augmented the production of IL-9 in primary MLR and Mls whereas the secretion of IL-3 was not or only marginally influenced. In contrast IL-2 had no effect on IL-9 synthesis during primary MLR and

2028902094

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Mls. IL-9 was measured with the aid of an IL-9 dependent T cell clone in the presence of neutralizing monoclonal anti-IL-4 (11B11) and anti-IL-2 (S4B6.1) antibodies. Biological activity of IL-9 was completely absorbed by a monoclonal anti-IL-9 (E6.M) antibody whereas IL-3, which was also present in the IL-9 containing supernatant, was not retained demonstrating the specificity of the bioassay and of the anti-IL-9 affinity column applied.

These data further support the notion that IL-4, beside its T-cell growth factor activity, can regulate the production of other lymphokines by T cells and in this way modulate their phenotypical development.

Institut für Immunologie, FU Berlin, Hindenburgdamm 27, 1000 Berlin 45, Germany

F.14 Expression of β-chain of interleukin-2 receptor (IL-2R) on various Hodgkin cell lines

H. Shao, O. Josimovic-Alasevic, and T. Diamantstein

Recent studies have shown that 10% of the peripheral blood lymphocytes express the β-chain of IL-2R (p70 IL-2R binding protein) constitutively. In this study we investigated whether various leukaemic cell lines express the β-chain. We found that Hodgkin cell lines L540, L428, KH-2 and CO, as well as the erythromyeloid cell line K562 constitutively express the β-chain of IL-2R on their surface, as detected by immunfluorescence staining with mAb specific for the β-chain and Northern blot analysis of mRNA isolated from these cells. In contrast, EBV-infected B-cell lines such as CESS, WEWAKE I and WEWAKE II do neither express membrane bound β-chain, nor a message for it.

Whether the constitutive expression of the IL-2R β-chain plays a role in the autonomous growth of the cells remains to be elucidated.

Institut für Klinische Mikrobiologie, Universität Erlangen, Wasserturmstr. 3, D-8520 Erlangen, FRG

F.15 *Leishmania major* is a potent inducer of interleukin-1α in splenic cells from parasite-infected mice, the activity of which is antagonized by interferon-γ

W. Solbach, H.-U. Beuscher, H. Wagner, and M. Röllinghoff

Mice of the strain BALB/c are extremely susceptible to an infection with *Leishmania major (L. major)* and usually succumb to the disease. In contrast, C57BL/6 animals completely recover from the infection and thus are resistent. Experimental evidence suggests that both resistance and susceptibility are mediated by functionally distinct $CD4^+$ T lymphocytes. Since IL-1 is an important cofactor for the activation of a subset of $CD4^+$ T cells, the expression of IL-1 activity in splenic adherent cells from *L. major*-infected susceptible and resistant mice was analysed after restimulation *in vitro*. IL-1 was measured by its costimulatory activity on the T_H2 cell line D10.G4.1. We found that 3 weeks after infection, cells form BALB/c mice produced substantially more IL-1 activity than those from C57BL/6 mice or from noninfected controls. These differences were due to an extensive accumulation of IL-1 producing cells in

2028902095

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

the spleens of infected BALB/c mice which were up to 12-fold higher as compared to C57BL/6 animals. More than 95% of the IL-1 bioactivity was mediated by IL-1α, as determined by blocking studies with antibodies. Co-incubation experiments using a mixture of a constant number of splenic adherent cells and varying amounts of syngeneic $CD4^+$ T lymphocytes revealed that the IL-1 activity was inhibited only by IFN-γ producing T cells. Such cells were found in resistant, but not in susceptible mice. The inhibitory action of IFN-γ could be completely antagonized with an anti-IFN-γ antibody. We conclude from this study that *L. major* is a potent stimulator of IL-1 activity, which in the absence of the counterbalancing activity of IFN-γ, may substantially contribute to the clonal expansion of disease-promoting $CD4^+$ T lymphocytes as seen in susceptible animals.

Institute of Immunology, Philipps University, 3550 Marburg, FRG

F.16 Stimulation of TNF-α synthesis by cyclic GMP

H. Sprenger, C. Koch, and D. Gemsa

Recently, we reported that TNF-α synthesis may be regulated by conspicuous changes of cyclic nucleotides. An intracellular increase of cyclic AMP displayed suppressive effects whereas elevated cyclic GMP was associated with stimulatory activities. For further studies, human monocytes were used as target cells for different cyclic GMP-raising compounds and TNF-α release was determined by bioassay of specific ELISA. A strong correlation between increase of cyclic GMP and subsequent TNF-α synthesis was found with the vasodilatating drug sodium nitroprusside which acted as strongly as extracellularly added cyclic GMP. Since sodium nitroprusside stimulates the soluble, cytoplasmic form of guanylyl cyclase, it was of interest to compare it to stimuli of the particulate, membrane-bound forms. We employed atrial natriuretic peptide (ANP) which directly binds to the membrane form of guanylyl cyclase as its receptor. Although ANP raised cyclic GMP in HeLa cells, it remained inefficient on monocytes and neither generated cyclic GMP nor TNF-α.

Apparently, ANP receptors were lacking or a coupling to the catalytic unit of guanylyl cyclase was missing on monocytes. The effects of lipopolysaccharides, strong inducers of TNF-α synthesis, were only inconsistently associated with an intracellular cyclic GMP generation but a strong potentiation occurred together with exogenous cyclic GMP. Taken together, the data support the notion that cyclic GMP may be one of the positive intracellular messengers for TNF-α production, but it still remains unclear whether all stimuli act via this pathway.

[1]Department of Immunology and Transfusion Medicine, Medical University of Hannover, 3000 Hannover, FRG, [2]Pharmaceutical Research, Ciba-Geigy Ltd., Basel, Switzerland

F.17 The human intracellular Mx-homologous protein is specifically induced by type-I-interferons

P. von Wussow[1], D. Jakschies[1], H. K. Hochkeppel[2], C. Fibich[1], L. Penner[1], and H. Deicher[1]

The murine Mx-2-protein is one of the best characterized IFN-induced proteins, which is necessary and sufficient to provide resistance of murine cells against influenza viral infection.

2028902096

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Recently the intracellular human protein homologous to the murine Mx-2-protein has been identified by means of a specific monoclonal antibody. The restricted induction of this intracellular protein in human mononuclear cells (MNC) by various cytokines was investigated.

MNC from 26 of 28 healthy people and 35 of 36 cancer patients before IFN-α therapy had no detectable Mx-homologous protein. Incubation of human MNC with IFN-α and IFN-β for 24 hours at different concentrations led to a dose dependent induction of the Mx-homologous protein. All IFN-α or IFN-β preparations tested were equally effective in eliciting this intracellular protein. IFN-γ induced only 1 % of the Mx-amount elicited by type-I-IFNs compared on a weight basis. Neither IL-1 nor IL-3, IL-4, IL-5, IL-6, TNF-α/β, G-CSF or GM-CSF at any of the concentrations tested were capable of eliciting any detectable amount of the Mx-homologue, while IL-2 was a poor Mx-homologous protein inducer. In the presence of high-titered IFN-α-antisera both IL-2 and IFN-γ were unable to stimulate this protein proving that IFN-γ and IL-2 indirectly induce the Mx-homologue via IFN-α. Therefore the human Mx-homologous protein is a strictly regulated protein in human PBL apparently only inducible by type-I-interferons.

[1] Institute for Molecular Pharmacology, Medical University Hannover, 3000 Hannover, FRG, and [2] Department of Molecular Genetics and Virology, The Weizmann Institute of Science, Rehovot, Israel

F.18 Changes in the types of TNF receptors during differentiation of HL-60 cells

Reinhard Winzen[1], David Wallach[2], Hartmut Engelmann[2], Klaus Resch[1], and Helmut Holtmann[1]

The cytokine tumor necrosis factor (TNF) can bind to two different types of cellular receptors. Employing antisera generated against the soluble forms of the receptors (Engelmann et al., J. Biol. Chem. 265:1531, 1990), it was asked what factors govern the expression of the two receptor types. The extent to which antiserum against each receptor type blocked the binding of (^{125}I)-TNF was taken as indicative of the amount of the respective receptor on the cell. The promyelocytic cell line HL-60 expressed both types of TNF receptors simultaneously. When differentiated along the granulocytic pathway by addition of retinoic acid, the ratio of type I versus type II receptors was only slightly changed, with some decrease in the relative amount of type I receptors. In contrast, after phorbol ester-induced differentiation along the monocytic pathway, type I receptors were no longer detectable, and binding of TNF could be ascribed to type II receptors only. Correspondingly, monocytes isolated from human peripheral blood expressed type II receptors mainly, whereas granulocytes carried comparable amounts of both receptor types on their surface. Immunoblot analysis of membrane preparations from monocytes, granulocytes and HL-60 cells (undifferentiated as well as differentiated by phorbol esters or retinoic acid) confirmed the results obtained in the binding assays. The results suggest that the expression of type I TNF receptors is selectively turned off during differentiation of myeloid precursor cells into monocytes.

2028902097

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Workshop G
Macrophages, Cellular Properties

Lehrstuhl für Medizinische Mikrobiologie und Immunologie, AG Infektabwehr, Ruhr-Universität Bochum, 4630 Bochum, FRG

G.1 Investigations of cellular interactions in allergic and inflammatory processes

R. Arnold and W. Konig

We investigated the effector capacity of inflammatory cells in cell-coincubation experiments. For this purpose polymorphonuclear granulocytes (PMN) and eosinophils were coincubated with mast cells, cells of the broncho-alveolar lavage (BAL), L929 cells or LMBs (lymphocytes, monocytes, basophils). Chemiluminescence, leukotriene generation and granular enzyme release were determined. By coincubation of PMN or eosinophils with mast cells as well as BAL cells the Ca-ionophore A23187 or opsonized zymosan induced chemiluminescence response was inhibited. By TCA-precipitation of the cell-supernatant of activated mast cells an increased chemiluminescence-inhibition was observed. Priming of eosinophils with IL-3 enhanced the enzyme release at the 1st and 2nd day of culture. The viability and the chemiluminescence were not affected. The eosinophils, mast cells and LMBs were capable of converting LTA4 to LTC4 without further stimulation. The coincubation of mast cells with eosinophils and eosinophils with LMB, respectively, resulted in a decreased LTC4 synthesis in the presence of Ca-ionophore and opsonized zymosan. Stimulation of coincubated PMN and eosinophils as well as PMN and L929 cells did not affect the lysozyme release compared to PMNs alone. In contrast, coincubation of PMN with mast cells or LMB decreased the lysozyme release. The release of β-glucuronidase in coincubated PMN eosinophil cell suspensions was diminished in the presence of different stimuli. These results support a potent modulatory role of effector cell-interactions in inflammatory processes.

Institute of Immunology, Philipps University, 3550 Marburg, FRG

G.2 Macrophages replicate influenza A virus and concomitantly produce tumor necrosis factor-α

A. Bender, F. Hinder, A. Schmidt, M. Nain, and D. Gemsa

Previously, we demonstrated that macrophages were target cells for influenza A virus infection. Macrophages synthesized virus proteins, released infectious particles and died. Virus

2028902098

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

infection was accompanied by a temporary macrophage activation as revealed by TNF-α gene expression and TNF-α protein release. This study was designed to correlate functional findings with morphologic evidence on the single cell level by using indirect immunofluorescence. Three types of rabbit antisera (AS) were used in a murine macrophage system: 1. AS against influenza A glycoproteins (anti-GP), 2. AS against complete virus preparations (anti-V), and 3. AS against murine TNF-α (anti-TNF-α). After influenza A virus infection, a homogeneous cytoplasmic staining was found with anti-GP. This staining appeared 4 h p.i. and markedly increased up to 8 hrs p.i. With anti-V, an additional fluorescence was detected in the perinuclear region. By using anti-TNF-α, a staining was found which was comparatively weak, similar to the one following exposure to nanogram amounts of lipopolysaccharide (LPS). However, when virus-infected macrophages were simultaneously stimulated with these low LPS concentrations, a strikingly enhanced intensity of immunofluorescence was observed with anti-TNF-α in the juxtanuclear region, most likely reflecting the Golgi apparatus. These data on the single cell level show that 1. influenza A virus is an inducer of TNF-α synthesis and 2. low LPS amounts potentiate virus-induced TNF-α production. Both findings, taken together, indicate that deleterious effects of combined influenza A/bacterial infections may be due, at least partially, to an enhanced TNF-α release.

Institut für Immunologie der Joh.-Gutenberg-Universität, D-6500 Mainz, FRG

G.3 Antigen-mediated cooperation between T cells and bone marrow-derived macrophages results in *de novo* Ia-synthesis in antigen-presenting cells

S. Frosch and A. B. Reske-Kunz

Bone marrow-derived macrophages (BMMΦ) obtained by *in vitro* culture of bone marrow stem cells in the presence of recombinant M-CSF are able to present antigen to distinct T cell clones, although these macrophages do not synthesize detectable levels of MHC class II molecules, as shown by Northern blot analysis, radiolabelling studies, and cytofluorometry. During cocultivation of T cells and macrophages for 24 hours T cells induced macrophage Ia synthesis in an antigen-specific manner by the secretion of Ia-inducing lymphokines. To find out which lymphokines may in principle be active in this cooperative process BMMΦ were pulsed with the recombinant lymphokines IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, TNF-α, TNF-β, IFN-γ, and GM-CSF for 24 hours, a duration which we have shown to be optimal, and the Ia-inducing capacity was analyzed. IL-6, TNF-α, and TNF-β, in addition to IFN-γ, GM-CSF, and IL-4, had potent Ia-inducing capacity. These results form the basis for devising inhibition studies using lymphokine-specific antibodies to reveal which lymphokines are actually active during the interaction between T cells and BMMΦ.

Supported by the Deutsche Forschungsgemeinschaft, SFB 311, Mainz.

2028902099

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Institute for Experimental Dermatology, University of Münster, Von-Esmarch-Straße 56, D-4400 Münster

G.4 Substance P-induced augmentation of allergic and irritant contact dermatitis in mice

M. Goebeler, J. Gutwald, and C. Sorg

The neuropeptide substance P (SP) has been shown to be involved in cutaneous inflammation. SP stimulates chemotaxis, margination, and endothelial adherence of leukocytes in postcapillary venules as well as proliferation of T lymphocytes and degranulation of mast cells.

We examined the influence of SP on allergic and irritant contact dermatitis in BALB/c mice. Ears were challenged with oxazolone or croton oil, respectively. Groups of mice were treated epicutaneously with 25 µl 10^{-5} M SP for a period of 4 days either during the sensitization or the elicitation phase. Ear swelling responses and biopsy specimens of challenged ears were evaluated in groups of SP-treated or control animals at 0, 8, 24, and 48 h after challenge.

Treatment with SP during the elicitation phase produced a significant enhancement of the ear swelling response throughout the first 48 h in both allergic and irritant contact dermatitis. SP showed no effect when applied alone or during the sensitization phase. Immunohistologically, the SP-treated mice showed a stronger early endothelial 7D10 (i.e. M1F) reaction and a more intense cellular infiltrate at 24 and 48 h than did untreated mice. Predominantly I-A^+-cells and, to a lesser extent, BM 8^+-cells (i.e. macrophages) could be observed.

We conclude that enhancement of allergic and irritant contact dermatitis by SP may be related to endothelial activation and stimulation of endothelial-leukocyte interactions. Our data provide evidence for a neurogenic modulation of immune response which might be important for contact dermatitis occurring in man.

Institute for Experimental Dermatology, University of Münster, Von-Esmarch-Straße 56, D-4400 Münster, FRG

G.5 Expression of intercellular adhesion molecule-1 (ICAM-1) and endothelial-leukocyte adhesion molecule-1 (ELAM-1) in evolving human contact dermatitis

M. Goebeler, J. Gutwald, C. Sunderkötter, and C. Sorg

Adhesion of blood leukocytes to vascular endothelium, a key step in the evolving inflammatory reaction, is promoted by endothelial surface glycoproteins such as intercellular adhesion molecule-1 (ICAM-1) and endothelial-leukocyte adhesion molecule-1 (ELAM-1).

Skin biopsy specimens of a volunteer were studied immunohistochemically for the expression of adhesion proteins and markers for mononuclear leukocytes at 0, 6, 24, 48, and 72 h after elicitation of allergic contact dermatitis with nickel chloride.

Normal skin showed staining for ICAM-1 restricted to dermal vascular endothelium, ELAM-1 could not be detected. Progressive staining for ICAM-1 was observed in endothelium as well as in perivascular cells between 24 and 72 h. ELAM-1 was not found before 24 h after challenge. It was strongly expressed by vascular endothelium of the dermal papillary layer at 24, 48, and 72 h after challenge. Infiltrating cells, mainly $CD3^+/CD5^+$ lymphocytes could be observed around ICAM-1^+ endothelium. A focal expression of ICAM-1 by keratinocytes, mainly in the basal epidermal

2028902100

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

layer, was noted at 48 and 72 h correlating with epidermal $CD3^+/CD5^+$ lymphocyte infiltration. Our results demonstrate that ICAM-1 and ELAM-1 are involved in dermal and epidermal leukocyte trafficking in human allergic contact dermatitis.

Institute for Experimental Dermatology, University of Münster, Von-Esmarch-Straße 56, D-4400 Münster, West-Germany

G.6 Allergens and irritants induce expression of adhesion molecules in cultured vascular endothelial cells

J. Gutwald, M. Goebeler, G. Meinardus-Hager, and C. Sorg

Vascular endothelium is increasingly appreciated as an active participant in evolving inflammatory reactions. Cytokines like interleukin-1 (IL-1) and tumor necrosis factor as well as bacterial endotoxin act directly on cultured vascular endothelium and induce activation antigens. We initiated a study to investigate the direct effect(s) of allergic (2,4-dinitrofluorobenzene (DNFB), 2,4-dinitrobenzene sulfonate ($DNBSO_3$), nickel chloride, chromium sulfate) and irritant (croton oil) substances on isolated endothelial cells. Cultured human umbilical vein endothelial cells (HUVEC) were incubated with the above mentioned substances for 10 minutes. Expression of intercellular adhesion molecule-1 (ICAM-1), endothelial-leukocyte adhesion molecule (ELAM-1), MHC class II antigens, and IL-1 were analyzed immunohistochemically 6, 24, and 48 h after stimulation. Both allergic and irritant substances induce an expression of ICAM-1 and ELAM-1 in HUVEC similar to activation by cytokines. Maximal staining for ELAM-1 was observed after 6 h, for ICAM-1 after 24 h. MHC class II antigens could not be induced in the first 48 h after stimulation. IL-1 was detected after 24 h.

Our results demonstrate that allergic and irritant substances act on endothelial cells. This may be relevant to the pathogenesis of human allergic and irritant contact dermatitis.

Institute for General and Experimental Pathology, University of Innsbruck Medical School, Fritz-Pregl-Straße 3, 6020 Innsbruck, Austria

G.7 Glucocorticoid-regulated transcripts in the mouse macrophage-like cell line P388D1 with potential significance for immunosuppression

A. Helmberg, R. Fässler, S. Geley, K. Jöhrer, G. Krömer, and R. Kofler

The molecular mechanisms of glucocorticoid-mediated immunosuppression are still poorly understood. To address glucocorticoid-regulated gene expression in cells of the immune system, a subtractive hybridization and cDNA cloning protocol was used to identify transcripts with glucocorticoid-dependent steady state levels. Two of the isolated transcripts lend themselves for a potential role in immunoregulation:

1. One upregulated transcript encodes the endogenous type-C retroviral envelope protein TM/p15E, for which various immunosuppressive effects have been shown, such as inhibition of lymphocyte proliferation, macrophage chemotaxis, and lymphokine production. The core amino acid sequence essential for these effects is conserved in homologous feline and human retroviruses.

2028902101

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

2. Nucleic acid sequence analysis of one down-regulated transcript revealed a 93 % match to the gene of syrian hamster 3-hydroxy-3-methylglutaryl-CoA reductase. Therefore, this cDNA clone probably corresponds to the as yet unknown mouse gene. The potential significance of this finding for immunoregulation derives from the fact that *in vitro* inhibition of lymphocyte HMG-CoA reductase results in decreased proliferative response to mitogens.

These results raise the possibility that the immunosuppressive activity of glucocorticoids might be mediated, in part, by regulating the expression of the above immunoregulatory proteins.

This work was supported by a grant from the Austrian Ministry for Science and Research.

[1]Institute of Immunology, Philipps University, Marburg, FRG, and [2]Division of Virology, Academy of Sciences, Jena, GDR

G.8 Coxsackie virus infects monocytes and induces release of tumor necrosis factor-α

A. Henke[1], A. Stelzner[2], M. Nain[1], and D. Gemsa[1]

Infections with coxsackie virus (CV), a positive single-stranded RNA virus, are associated with an inflammatory response in which the leukocyte components have received little attention. Since presumptive evidence has indicated that macrophages may play a central role, we designed a study to examine the susceptibility and reactivity of human monocytes in coxsackie infection. Purified monocytes were exposed to CV, strain B3, at a multiplicity of infection (MOI) of 0.1 to 20, and viability, adherence, virus production, and release of interferon-α/β (IFN-α/β) and tumor necrosis factor-α (TNF-α) were determined 12 and 24 h after infection. CV infection led to an increased adherence and spreading with a maximum between 2 to 10 MOI. Monocytes were capable of supporting virus replication: an up to 50–100 fold virus multiplication was noted which occurred without apparent loss of viability. As for IFN-α/β, CV-infected monocytes were potent producers of TNF-α which was present 12 hrs after infection, increased up to 24 hrs, was inducible by as little as an MOI of 0.1 and was maximal between an MOI of 5 to 10. These findings indicate that monocytes are particularly sensitive to CV infections since they not only support virus replication, but also respond with potential defense mechanisms such as release of IFN-α/β and TNF-α. It remains to be elucidated which role TNF-α may play in the immunopathology of CV infections.

Abteilung Klinische Immunologie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Straße 8, 3000 Hannover 61, FRG

G.9 Characterization of CD16⁻ natural killer (NK) cells in peripheral blood of bone marrow transplant patients

R. Jacobs, R. Leo, G. Hinrichs, A. Kemper, P. Uciechowski, M. Stoll, and R. E. Schmidt

In order to investigate human NK cells within an altered immune system, we studied peripheral blood lymphocytes in patients (pts) after bone marrow transplantation. Earlier

2028902102

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

investigations concerning NK-cells in pts during interleukin-2 (IL-2) therapy and observations in children with immune deficiency syndromes revealed in addition to normal NK cells a new subgroup of $CD3^-$ $CD56_{bright}^+$ $CD16^-$ NK cells. NK cells in healthy volunteers are $CD3^-$ $CD56_{dim}^+$ $CD16^+$. Analogous to this finding we detected a distinct NK cell population in pts in the early phase of immune reconstitution. These cells were extensively characterized by cytofluorometric methods and they are found to be $CD3^-$ $CD56_{bright}$ $CD16^-$. Moreover this NK subgroup coexpresses a broad panel of activation antigens. Some of these proteins, which are exclusively expressed on this NK subpopulation but not on normal NK cells are CD26, CD2R and the p75 chain of the IL-2 receptor. None of both NK cell types were positive for the CD25 antigen. Cell separation using FACS revealed $CD56_{bright}^+$ and $CD56_{dim}^+$ as equal potent lymphokine (i.e. IFN-γ and TNF-α) producers. But they clearly differed in their NK and ADCC activities. This new NK subset appearing under maximally activated *in vivo* conditions might be a precursor for mature peripheral blood NK cells.

[1]Department for Immunology, University of Kiel, Brunswikerstraße 4, D-2300 Kiel 1, FRG, and [2]Ludwig Institute for Cancer Research Stockholm Branch, Stockholm, Sweden

G.10 Influence of *myc* oncogene transfection on the MHC-alloimmunogenicity of the mouse macrophage cell line J774A1

H. Kostka[1], H.-U. Wottge[1], B. Seliger[2], and W. Müller-Ruchholtz[1]

It has been shown that transfection of the mouse macrophage cell line P388D1 with the *v-mos* oncogene can lead to complete downregulation of its *in vitro* allostimulatory capacity. Since the transforming potential of the *mos* oncogene prevents *in vivo* studies on the tolerogenic potential of these cells, we studied whether transfection of the BALB/c derived macrophage cell line J774A1 with the non-transforming *myc* oncogene might reveal similar downregulating effects.

Methods: J774A1 was transfected with the *v-myc* oncogene using a retroviral vector. After cloning the cells were tested as stimulators for MHC-different C3H spleen cells *in vitro*.

Results: (1) None of the first 12 *myc*-transfected clones showed downregulated allostimulatory capacity compared to normal BALB/c macrophages. (2) This contrasts with 2 out of 2 *mos*-transfected macrophage clones which showed downregulation without any reduction of MHC class I and II cell surface expression. (3) The continuing analysis will provide more information on parameters which determine oncogene-dependent downregulation of alloimmunogenicity.

Conclusions: (1) Oncogene transfection allows the establishment of MHC high expressor, nonimmunogenic cell clones, though predictions on the outcome of a single oncogene transfection is not yet possible. (2) In the context of our previous results it is postulated that manipulations of immunogenicity of cells require better knowledge on the relevant insertion points in the genome.

2028902103

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Clinical Research Unit for Rheumatology at the University of Erlangen-Nürnberg, Schwabachanlage 10, 8520 Erlangen, FRG

G.11 Isolation of cDNA clones differentially expressed in stimulated monocytoid cell lines

K. Kühn, W. Bertling, and F. Emmrich

The investigation of gene expression patterns of monocyte-like cell lines like U937, HL 60 and THP-1 may contribute to the understanding of the molecular basis of monocyte/macrophage differentiation and activation pathways. We chose to investigate the effects of an activation signal (IFN gamma) in combination with the differentiation signal dihydroxy-vitamine D3 (DVD3) for the human monocytoid cell line U937. A cytofluorometric analysis showed that DVD3 is more potent than IFN gamma in stimulating the expression of differentiation linked surface antigens like HLA class II, Fc-receptor or complement receptor. Combination of the two stimuli did not reveal a positive synergistic effect. For some antigens expression levels were even slightly lower than with DVD3 alone. The RNA from two and four days IFN gamma/DVD3 stimulated U937 cells was taken to construct a cDNA library which was differentially screened in order to obtain clones representing mRNA's induced in stimulated cells. Several clones were isolated showing enhanced expression either in stimulated U937 cells or stimulated HL 60 cells as demonstrated by Northern blot experiments. Two of the clones induced in U937 show comparable mRNA induction levels in IFN gamma and double stimulated cells. Expression after 4 days of stimulation was reduced as compared to two day stimulation. In contrast, stimulation with DVD3 alone did not induce the corresponding mRNA's suggesting that the induction in double stimulated cells was due to the action of IFN gamma alone. Partial sequencing and comparison with gene data bases revealed for one of the clones significant homology to the chicken progesteron receptor and the human *v-erbA* gene suggesting a relation to the family of steroid receptors. The results obtained support our idea that cDNA-clones isolated from stimulated monocytoid cell lines may be taken as probes for the characterization of monocyte/macrophage gene expression patterns related either to differentiation or to activation.

Department of Molecular Pharmacology, Medical School, 3000 Hannover, FRG

G. 12 Characterization of cytostasis and TNF bioassay using the human monoblastoid cell line U937

C. A. Mittermair, H. H. Radeke, and K. Resch

Usually tumor cytostasis and TNF-bioactivity are measured using the mouse target cells P815 and L929. We now established a method with the human monoblastoid cell line U937. Cytolytic activity was determined in a "short time" assay (6 h) and cytostatic/-lytic effects in a "long time" assay (48 h). For the 6 h-assay 1×10^5 cells/well were plated in 96-well-plates and dilutions of several recombinant cytokines in medium with 5 % FCS (± cycloheximide (CHI), 1 µg/ml) were added. Following the incubation period U937 were stained with MTT for additional 1.5 h. The 48 h-assay was performed with an initial cell density of 2×10^4 cells/well, cytokines were added in medium with 5 % FCS without CHI. After 46 h [^{3}H]-thymidine incorporation was measured during the last 2 h. In the 6 h-assay with CHI the lysis of the

2028902104

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

target cells correlated with MTT-staining. rhTNF-α induced lysis of U937 cells (50 % with 10 U/ml = 1 ng/ml) in a concentration dependent way. Lymphotoxin (LT, rhTNF-β) was less effective causing 60 % cell lysis at 1000 U/ml (= 10 ng/ml). Other cytokines like IL-1α and β, IFN-gamma and IL-6 had no significant effects on target cells. In the 48 h-assay MTT-staining correlated well to [^{3}H]-TdR incorporation, nevertheless using [^{3}H]-TdR pulses in the absence of CHI markedly increased the sensitivity. In this 48 h-assay again TNF-α had a marked antiproliferative activity (50 % with 100 U/ml = 10 ng/ml), however, the cells were more sensitive to LT (50 % with 10 U/ml = 0.1 ng/ml). Moreover, rhIL-6, ineffective in the "short time" assay, had a higher growth inhibitory effect than TNF-α (50 % with 10 U/ml rhIL-6 = 0.5 ng/ml). All other cytokines tested had no or only little effect on the proliferation of U937, only IFN-gamma caused a reduction of 25 % at a concentration of 1000 U/ml (100 ng/ml).

Preliminary experiments with stimulated human peripheral blood monocytes showed 50 to 60 % inhibition in the 6 h-assay, whereas in the 48 h-assay the supernatants reduced the [^{3}H]-TdR incorporation up to 99 %. Thus, in conclusion, this U937 assay not only provided us with a very sensitive method to determine TNF-bioactivity, but in addition employing the respective antisera raises the possibility to discriminate TNF effects from other cytostatic factors generated by human cells.

Supported by a grant from BASF.

Institute of Immunology, University of Marburg, FRG, and Pulmonary Unit, Department of Medicine, University of Vermont, Burlington, U.S.A.

G.13 Inhaled silica particles induce a persistent stage of macrophage preactivation

C. Mohr, C. Graebner, D. R. Hemenway, G. S. Davis, and D. Gemsa

Silicosis is a chronic and progressive lung fibrosis caused by inhalation of silica particles. It has been proposed that a persistently stimulated immune system may ultimately lead to pulmonary fibrosis. In the present study, we were interested in the secretion of TNF-α from silica-laden alveolar macrophages (AMΦ) since this cytokine may be involved in pulmonary inflammation, granuloma formation, and fibrosis. 4 to 6 months after silica exposure, AMΦ were harvested from Fischer 344 rats. It was found that AMΦ from silica-dusted rats displayed an extreme sensitivity to ng amounts of LPS and rapidly secreted high concentrations of TNF-α when compared to AMΦ from sham-exposed control rats. Peritoneal macrophages (PMΦ) without direct contact to inhaled silica particles displayed a similar TNF-α response, indicating a systemic activation of MΦ. To examine TNF-α synthesis regulating arachidonic acid metabolites, the inhibiting prostaglandin E_2 (PGE_2) and the stimulating leukotrienes were examined. The release of PGE_2 was elevated whereas leukotrienes were decreased, suggesting that a deficient regulation of TNF-α synthesis may prevail in silica-preactivated macrophages. The mechanism of silica-induced, systemic MΦ preactivation still remains unknown, however, evidence is accumulating that lymphocytes may be involved.

2028902105

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

Department of Immunology, Department of Internal Medicine, Medical University of Hannover, 3000 Hannover 61, FRG

G.14 Increased number of monocyte precursor cells (M-CFC) in bone marrow, spleen, and liver of autoimmune BXSB-mice

G. Vieten, A. Olp, S. Heilkantner, M. Fricke, K. Hartung, and H. Deicher

BXSB-mice develop an autoimmune disease which resembles human SLE. During their lifetime male BXSB-mice develop an increasing monocytosis in the peripheral blood which is unique among lupus mice. Our studies were undertaken to investigate whether an alteration at the bone marrow (BM) stem cell level may be the cause of this monocytosis. We undertook serial BM examinations employing a standard soft agar stem cell assay. We observed that BM of BXSB-mice contains a considerably higher number of M-CSF reactive macrophage precursor cells than all other mouse strains tested. Furthermore, we observed a continuous increase of M-CFC during the lifetime of BXSB-mice, concomitant with the progressing disease and the developing monocytosis in the peripheral blood. The number of M-CFC in male BXSB-mice was significantly higher than in females. At the age of 8 weeks male BXSB-mice produce twice as much M-CFC in the spleens than females and at the age of 20 weeks seven times more than females. In addition, in the liver we found progressive increase of M-CFC during the lifetime of male BXSB-mice. The increase of M-CFC might be one reason for the monocytosis in the blood and possibly for the rapid progression of the disease in BXSB male mice.

Supported by DFVLR 01 VM 8606/3.

Department of Medical Microbiology and Immunology, University of Ulm, Albert-Einstein-Allee 11, D-7900 Ulm, FRG

G.15 65 kD heat shock protein (hsp 65) epitopes are expressed on the surface of mouse macrophages

Angela Wand-Württenberger, Bernd Schoel, and Stefan H. E. Kaufmann

Different types of heat shock proteins (hsp) are expressed in special subcellular locations. For the hsp 65 of mammalian cells (called P1), this was found to be the mitochondrial matrix. So it was surprising for us to find this molecule (or its homologue) on the cell surface. We investigated the expression of hsp 65 in mouse bone marrow derived macrophages (BMM) following activation by m-IFN-γ and/or infection with *M. bovis* BCG. FACS analysis and fluorescence microscopy was performed to look at the expression of hsp 65 epitopes detected by the anti-hsp-65 mAb ML30 (made against hsp 65 of *M. leprae* (amino acids 276–297), also staining P1 (amino acids 301–321), kindly provided by Dr. J. Ivanyi) and rabbit-anti-hsp-65 IgG (made against purified recombinant hsp 65 of *M. bovis* BCG). Surface expression was observed in all the cells. Induction could only be detected with IFN-γ activation while BCG infection alone had no significant effect. With IFN-γ activation before infection enhanced surface expression was also seen. Labeling of the surface with ^{125}I and subsequent immunoprecipitation with rabbit-anti-hsp65-IgG yielded a single band of 60 kD in the autoradiogram. These data show that BMM carry structures on their surface which share properties of the ML30 epitope (301–322) and epitopes shared with hsp 65 of *M. bovis* BCG (stained with rabbit-anti-hsp65-IgG) – another support of the notion that molecules (or homologues) of hsp 65 might be translocated to the cell surface.

2028902106

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000

[1]Institut für Mikrobiologie, Technische Hochschule Darmstadt, FRG, and [2]Immunpharmakologie, Hoechst AG, Werk Kalle-Albert, Wiesbaden, FRG

G.16 Outer membrane protein as immunomodulator of the interaction of lipopolysaccharide with macrophages

G. Weber[1], D. Heck[2], R. R. Bartlett[2], and K. Nixdorff[1]

The various components of the outer membrane of Gram-negative bacteria are tightly bound to one another in the native structure. The immune system is often confronted with complexes of these components in addition to single constituents. Our previous studies have shown that an outer membrane protein (39 kD protein) from *Proteus mirabilis* is a strong modulator of the LPS-specific, T cell-dependent, IgG antibody-producing cell responses in mice. In the present report we have investigated possible immunomodulating effects of this protein at the level of LPS-macrophage interaction. When complexed with LPS, the 39 kD outer membrane protein completely inhibited the strong LPS-induced, lucigenin amplified chemiluminescence of both peritoneal and bone marrow-derived macrophages of mice. Furthermore, it abrogated the LPS-mediated suppression of interferon gamma-induced exposition of MHC II on the murine macrophage surface as determined in an enzyme-linked immunosorbent assay (ELISA) using monoclonal rat antibody specific for mouse MHC II haplotype H-2^b. Studies are underway to examine the possible modulating effects of the 39 kD protein on other parameters of LPS activation of macrophages, including cytotoxic action against L929S target cells as well as the production of interleukin-1 and arachidonic acid metabolites.

Department of Immunology, University Göttingen, D-3400 Göttingen, FRG

G.17 Late differentiation of long term cultured human macrophages

H. Xu and J. H. Peters

The differentiation of monocytes into macrophages is a long process involving several steps of changes in morphology, function, biochemistry, and phenotype. In this report we have used a panel of mAb to study this process: TIB228 (CD14); X-4, X-14, and X-15 recognizing macrophages; X-11 reactive with terminal complement complex (TCC) and X-12 reported by us to recognize interdigitating dendritic cells. The results showed that monocytes, when cultured in the medium RPMI-1640 with 10 % normal human serum (NHS) for up to 5 weeks, underwent differentiation into macrophages and exhibited different surface features associated with different differentiation stages. Fresh monocytes (day 0–1) expressed CD14 antigen but no macrophage markers as identified by our mAbs X-4, X-14, and X-15. At day 3, the cultured monocytes acquired the antigens for X-4, X-14 (70–80 %), and X-15 (60–70 %) as determined by FACS-analysis. The expression of the X-14 and X-15 antigens on cultured macrophages, compared with that of CD14 antigen, did not reach 100 %, as followed to 5 weeks by immunperoxidase staining. Starting at 2 weeks of culture, the macrophages, cultured either with 10 % NHS or FCS, expressed TCC on their surface without being killed. Surprisingly, the macrophages cultured for more than 3 weeks, either in 10 % NHS or FCS, expressed the antigen recognized by X-12 which stained only about 1–2 % cells of fresh or two weeks cultured monocytes under the same conditions. Thus, the expression of this antigen on long term macrophages implies a link between monocytes and interdigitating dendritic cells.

2028902107

Source: https://www.industrydocuments.ucsf.edu/docs/lfdl0000